

WHAT IS CLAIMED:

1. A recombinant hepatitis B surface antigen (rHBsAg) having an *in vitro* relative potency of at least 2.5.
- 5 2. The rHBsAg of Claim 1 wherein the *in vitro* relative potency is at least 3.0.
3. The rHBsAg of Claim 1 wherein the *in vitro* relative potency is
- 10 at least 3.5.
4. The rHBsAg of Claim 1 wherein the *in vitro* relative potency is at least 4.0.
- 15 5. The rHBsAg of Claim 1 wherein the protein is expressed in a host selected from the group consisting of yeast, *E. coli*, insect and mammalian host cells.
6. A vaccine comprising a therapeutically effective amount of the
- 20 rHBsAg of Claim 1.
7. The vaccine according to Claim 6 further comprising a therapeutically effective amount of at least one antigen selected from the groups consisting of Hepatitis A virus, *Varicella zoster*, *Neisseria meningitis* outer membrane protein, *Streptococcus pneumonia* capsular polysaccharide, Diphtheria toxoid, Tetanus toxoid, polyribitol phosphate, whole cell pertussis, a-cellular pertussis, and polio.
- 25 8. A method of making recombinant hepatitis B surface antigen (rHBsAg) comprising:
 - a) providing sterile filtered rHBsAg purified from a cell culture,
 - b) adding a redox buffer to the rHBsAg,
 - c) adjusting the temperature to from about 34°C to about 38°C,
 - d) incubating the rHBsAg at about 34°C to about 38°C for about 40 to
 - 30 about 240 hours.
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9. The method of Claim 8 wherein step c is performed before step b.

10. The method according to Claim 8 wherein the redox buffer comprises thiol compounds selected from the group consisting of thiol compounds having a MW less than about 1000 Da and the corresponding disulfide compounds.

11. The method according to Claim 10 wherein the redox buffer is a mixture of at least one thiol compound and at least one disulfide compound.

12. The method according to Claim 11, wherein the ratio of thiol compound to disulfide compound is between about 30:1 and about 1:1.

13. The method according to Claim 12 wherein the concentration of thiol compound is between about 0.05 mM and about 5.00 mM.

14. The method according to Claim 13 wherein the ratio of glutathione to oxidized glutathione is selected from the group consisting of about 20:1, about 10:1, about 10:4, about 5:1, about 2:1 and about 1:1.

15. The method according to Claim 13 wherein the thiol compound is glutathione and the disulfide compound is oxidized glutathione.

16. The method according to Claim 15 wherein the concentration of glutathione is about 1.0 mM and the concentration of oxidized glutathione is about 0.2 mM.

17. The method according to Claim 8 further comprising the steps of

- e) adding an aluminum adjuvant, and
f) co-precipitating the rHBsAg and the adjuvant.

18. The method according to Claim 8 further comprising the steps of

- e) adding about 0.01% final concentration of formalin ,

f) incubating the rHBsAg at from about 34°C to about 38°C from about 40 to about 72 hours,

wherein the incubation in step d is from about 40 to about 190 hours.

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19. The method according to Claim 17 further comprising the steps

of

g) adding an aluminum adjuvant, and

h) co-precipitating the rHBsAg and the adjuvant.

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20. The method according to Claim 17 wherein the incubation in step d is about 60 hours and the incubation in step f is about 40 hours.